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Originals

Morphological Appearance of Cultivated *Senna tora* (Caesalpiniaceae) from Sri Lanka

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In 1993 and 1994 we obtained two types of seeds of *Senna tora* (L.) Roxb. from Sri Lanka, which showed a considerable difference in size, and also exhibited morphological differences when cultivated. To clarify their morphological characteristics in detail and to elucidate their genetic relationship, a cultivation study and allozyme analysis was carried out. It was apparent that there were two strains of *S. tora* in Sri Lanka, which showed morphological differences (they are referred to in this report as type A and type B). The results of allozyme analysis showed that *S. tora* type A, type B, and *S. obtusifolia* could be distinguished by the difference in the allele of AAT-2, and also that *S. tora* (both types A and B) and *S. obtusifolia* could be distinguished by the difference in the alleles of SKD and AAT-1.

Key words: allozyme analysis, morphology, *Senna tora*, Sri Lanka.

In Sri Lanka the leaves and stems of *Senna tora* (L.) Roxb. (= *Cassia tora* L.) (Caesalpiniaceae) have been used as a laxative for the therapy of habitual constipation and haemorrhoids, and its seeds have also been used as an anti-parasitic for the therapy of ringworms and scabies (Jayaweera 1981). In Japan the seeds of *S. tora* and *Senna obtusifolia* (L.) H.S.Irwin & Barneby have been used as a crude drug –"Ketsumeishi"– for flatulence. On the Japanese market there are two types of "Ketsumeishi" which have different sizes and shapes – the smaller one has been said to be derived from seeds of *S. tora* (Iwasa 1956, Akiyama et al. 1996).

In 1993 and 1994 we obtained two types of seeds of *S. tora* in Sri Lanka, which showed a considerable difference in size (Fig. 1). The resultant plants also exhibited morphological differences when cultivated, in 1995 (Fig. 2). To clarify their morphological characteristics in detail, the two types of *S. tora* were cultivated and observed, using *S. obtusifolia* as a reference. In addition, in order to clarify their genetic relationship, an allozyme analysis was carried out. In this paper their morphological and zymographic differences are reported.

Materials

1. *Senna tora* (L.) Roxb. type A (Fig. 1-left, collected at Haldummulla Ayurvedic Herbarium, Beragalaory's Road, Haldummulla, Sri Lanka, 1993. 9).
2. *Senna tora* type B (Fig. 1-right, collected at Bandaranaike Memorial Ayurvedic

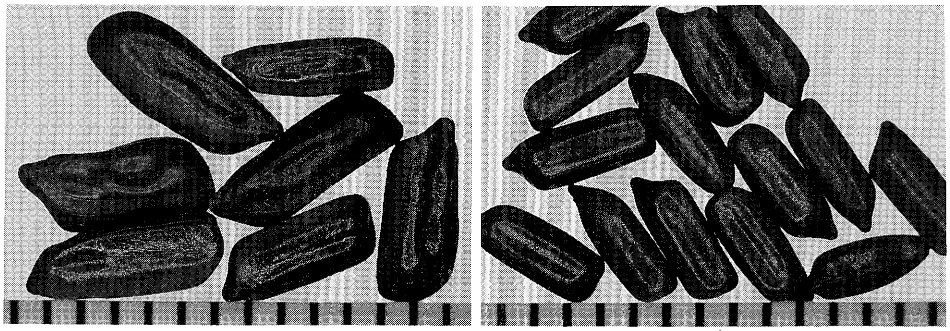


Fig. 1. Difference in seed size of *Senna* materials collected from Sri Lanka. *S. tora* type A (left) and *S. tora* type B (right).

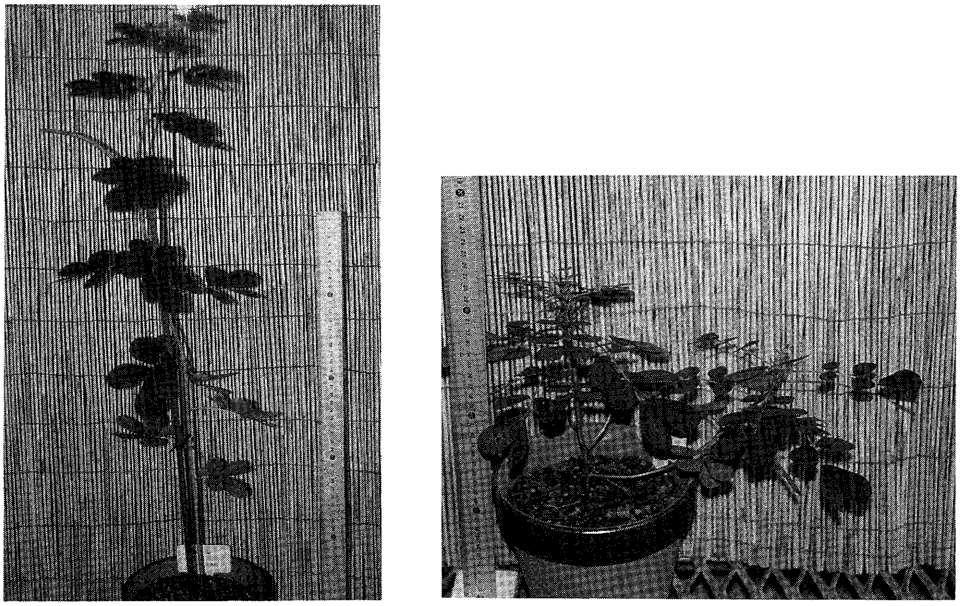


Fig. 2. Difference between *Senna tora* type A (left) and *S. tora* type B (right) in gross morphology.

Research Institute, Nawinna, Maharagama, Sri Lanka, 1994. 10).

3. *Senna obtusifolia* (L.) H.S.Irwin & Barneby (collected at Tokyo Metropolitan Medicinal Plant Garden, Kodaira, Tokyo, Japan, 1992).

Methods

Cultivation and Observation

Fifteen seeds of each type were sown in the greenhouse of the Medicinal Plant Garden, Showa Pharmaceutical University, Machida, Tokyo, on May 13, 1996 with 10 cm distances between each, and were observed.

Isozyme Electrophoresis

Zero-point-five gram of fresh leaves were cut into pieces and ground in ca. 1 ml of extraction buffer consisting of 0.14 M NaCl, 0.02 M NaOH, 0.12 % dithioerythritol, 0.1 % Dowex (HCl), a small amount of polyvinylpyrrolidone, and 4 % 2-mercaptoethanol. The extract was centrifuged at 15,000 rpm for 15 min at 4 °C. The supernatant was filtrated following Nishino and Morita (1994) with slight modification. The filtrate was electrophoresed using vertical slab equipment with 7.5 % polyacrylamide gel. Electrophoresis was carried out at 30 mA, 300 V per gel for ca. 100 min at 4 °C. Aspartate aminotransferase (AAT) and Shikimate dehydrogenase (SKD) were stained by the standard method.

Results

Morphology

(1) Growth and the Period of Flowering (Fig. 3):

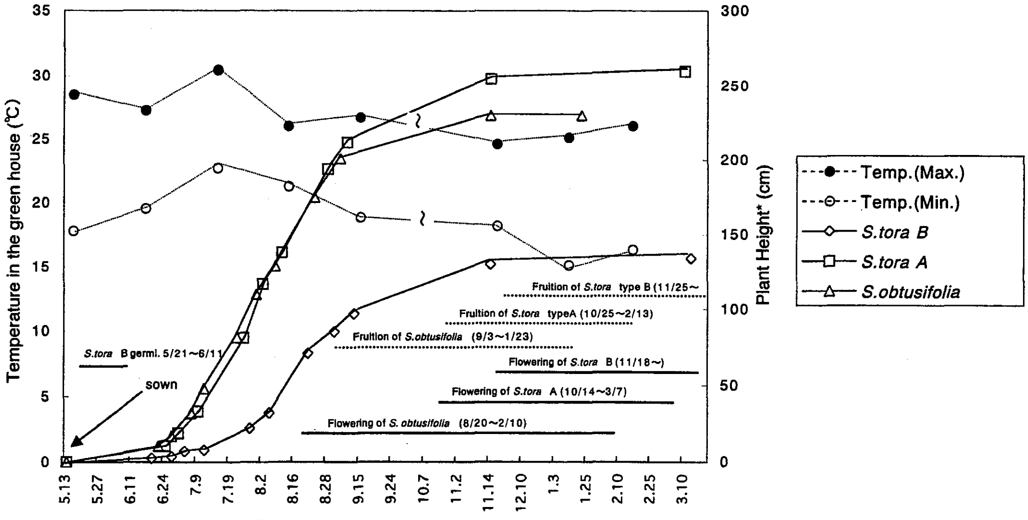
The growth in height of the plants began about one month after sowing, and stopped in early November (five months after sowing). The period of flowering and bearing fruits was different between the materials. *Senna obtusifolia* began flowering from the 31st August, *S. tora* type A one and half months later (from 14th October), and *S. tora* type B a further month after this (from 18th November).

(2) Plant Height and Branching (Table 1 and Fig. 4):

Senna tora type A reached a height of almost 3 m, which is more than two times taller than *S. tora* type B, and also taller than *S. obtusifolia*. *Senna tora* type B was the smallest of all. The branching pattern was different between the materials (Fig. 4). *Senna tora* type A had branches concentrated at the upper part of the main stem, while *S. tora* type B had them at the lower part. The branches were spreading at right angles in *S. tora* type A and B, whilst those of *S. obtusifolia* were more vertically inclined.

(3) Leaf, Flower and Fruit (Table 1):

Leaf: Rachises of the leaves of *S. tora* type A and *S. tora* type B were longer than those of *S. obtusifolia*. The leaflets of *S. tora* type A were the longest of all, and those of



*) The value of the average indivisual of each group was shown.

Fig. 3. Period of growth stages in the three *Senna* plants and their height.

Table 1. Characteristics of three materials of *Senna*. Where multiple values are given, these are minimum, mean and maximum, respectively

			S. tora type A	*S. tora* type B	*S. obtusifolia*
Plant height (cm)			180–240–285	40–76–130	150–204–250
Leaf	length of leaflet[1] (cm)	Nos. 6–15[2]	4.8–7.3–8.2	5.0–6.0–7.3	5.0–7.0–7.8
		Nos. 16–25	8.0–8.4–8.9	4.4–5.3–6.6	4.9–6.9–8.4
	length of rachis (cm)	Nos. 6–15	6.3–11.2–13.4	4.5–9.8–12.8	5.3–5.4–6.5
		Nos. 16–25	10.0–11.8–12.5	9.4–10.0–11.8	4.0–7.4–9.2
	stipule	length (cm)	1.3–2.2–2.5	1.0–1.4–1.7	1.0–1.2–1.3
		width (mm)	4.1–5.1–7.0	4.1–5.7–7.0	9.0–10.8–12.4
Flower	position of inflorescens		terminal	terminal	axil
	length of peduncle (cm)		0.7–0.8–0.9	0.5–0.6–0.7	1.7–2.0–2.2
	shape of apex of anther		obtuse	obtuse	cuspidate
	size of pollen grains		not uniform	not uniform	uniform
	percentage of stained pollen in aceto-orcein (%)[3]		8.2–48.5–83.1	41.5–46.9–50.0	91.8–92.9–93.4
Fruit	length of peduncle (cm)		0.6–0.9–1.0	1.3–1.6–1.9	1.8–2.1–2.5
	length of fruit (cm)		4.3–8.0–11.3	3.1–7.9–14.3	5.5–9.3–13.9
Chromosome number (2n)			26	26	26
Zymogram pattern	AAT-2		c	b	a
	AAT-1		b	b	a
	SKD		b	b	a

[1] One of the terminal leaflet pair was used to measure the size.
[2] The numerals show the leaf number counted from the base.
[3] More than 500 pollen grains were counted per anther.

S. tora type B were shorter than that of *S. obtusifolia*. The stipules of *S. tora* (both types A and B) were narrower than those of *S. obtusifolia*.

Flower (Fig. 5): *S. tora* (both types A and B) had flowers near the terminal of the branches. *Senna obtusifolia* had flowers mainly at the main axils. The shape of pollen grains of *S. tora* (both types A and B) differed in size, while those of *S. obtusifolia* were uniform (Fig. 6). The percentage of aceto-orcein stained pollen grains is 48.5 in *S. tora* type A, 46.9 in type B, and 92.9 in *S. obtusifolia*, respectively.

Fruit (Table 1): The fruits of *S. tora* type B were narrower than those of the other specimens and had a remarkable suture. The stipes of the fruits of *S. obtusifolia* were the longest, and those of *S. tora* type A were shortest.

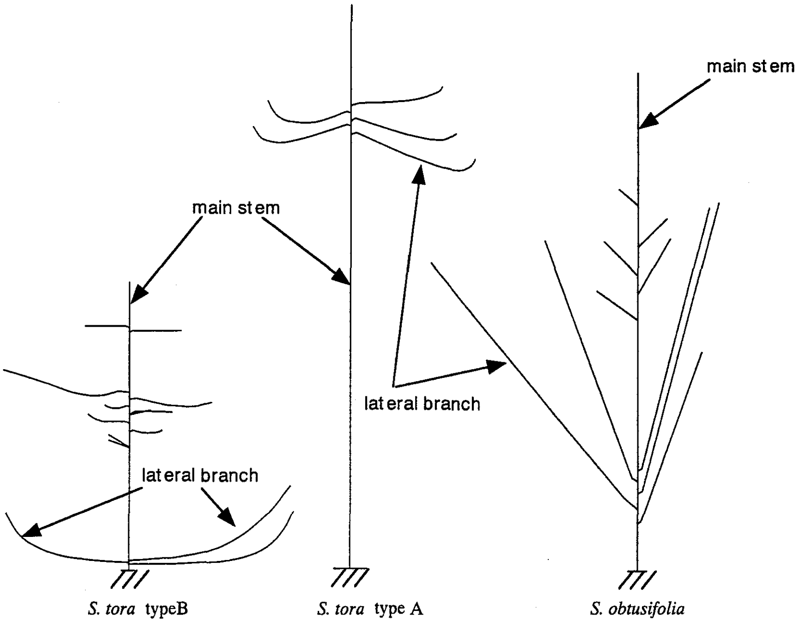


Fig. 4. Typical branching patterns of the three *Senna* materials.

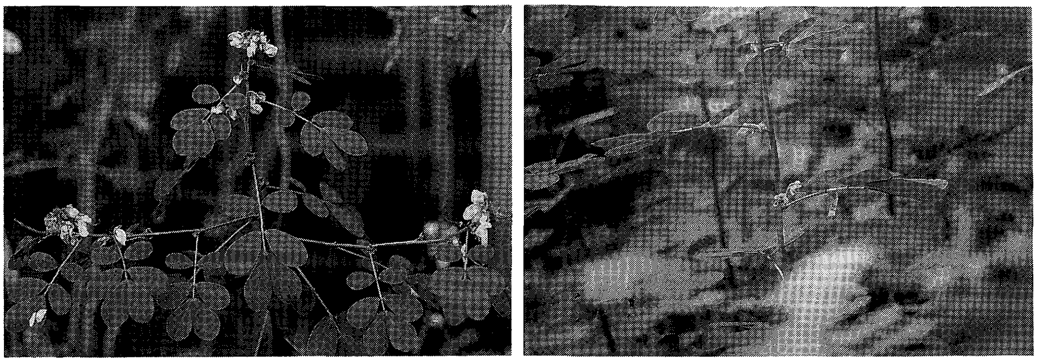


Fig. 5. Difference in the position of inflorescence between *Senna tora* type A (left) and *S. obtusifolia* (right).

(4) Gland (Fig. 7):

Firstly, the position of glands on the leaves could be divided into three types. They are referred to in this report as a-pattern, b-pattern, and c-pattern respectively and shown in Fig. 7. Most of the early leaves of all three materials had 4–5 leaflets and 1 gland (Fig. 7, a-pattern). From the sixth leaf and up, the leaves commonly had six leaflets and 2 glands in *S. tora* (Fig. 7, c-pattern), while those of *S. obtusifolia* had 1 gland at the basal pair of leaflets (Fig. 7, b-pattern). Some leaves on the branch of *S. tora* type B showed only 1 gland but it occurred at the

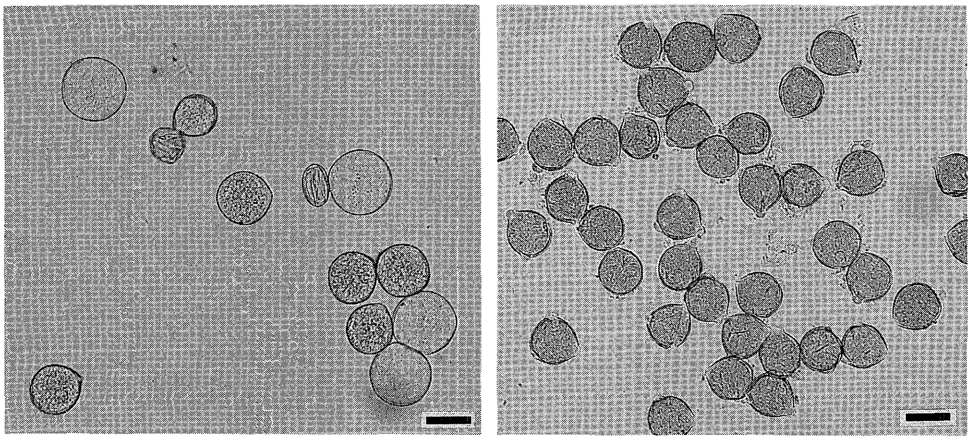


Fig. 6. Pollen grains of *Senna tora* type A (left) and *S. obtusifolia* (right). Scale bar = 30 μm.

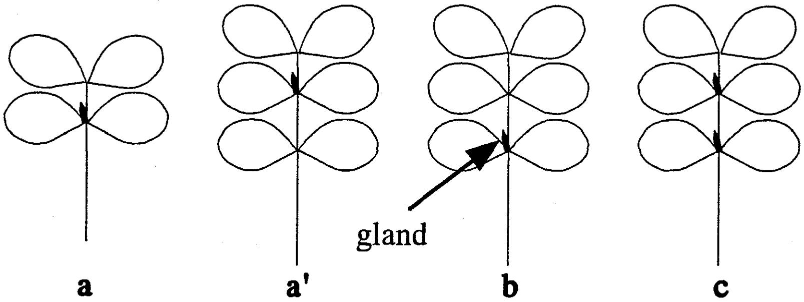


Fig. 7. Patterns of the position of glands on leaves of the *Senna* materials. **a**: pattern commonly found at juvenile stage, **a'**: pattern occasionally found in *S. tora* type B, **b**: pattern commonly found in *S. obtusifolia*, **c**: pattern commonly found in both types A and B of *S. tora*.

middle pair of the leaflets (Fig. 7, a'-pattern). Accordingly, *S. tora* and *S. obtusifolia* could be distinguished by the difference in the number and position of glands.

(5) Seed (Table 2):

Materials before sowing: The shape of the seeds of *S. tora* (both types A and B) is columnar (Fig. 1), and that of *S. obtusifolia* is oblong. The seeds of *S. tora* type A are larger than those of *S. tora* type B.

Products of the cultivation in 1997: The harvested seeds of three materials were larger than those initially sown in each case. However, the morphological characteristics and relative size of the three materials had not changed. The weight of 100 harvested seeds of each materials was 3.65 g (*S. obtusifolia*), 2.34 g (*S. tora* type A), and 1.61 g (*S. tora* type B), respectively.

Allozyme Analysis

SKD (Fig. 8): The patterns of the bands were divided into two types. One was *S. tora* (both types A and B) type, and the other was

Table 2. Size and weight of seeds of three materials of *Senna*

	S. tora type A				*S. tora* type B				*S. obtusifolia*			
	width (mm)	length (mm)	weight (g/100 seeds)	yield (g/plant)	width (mm)	length (mm)	weight (g/100 seeds)	yield (g/plant)	width (mm)	length (mm)	weight (g/100 seeds)	yield (g/plant)
Before sown	1.42	3.94			1.35	3.79			2.22	4.24		
	2.18	5.42	1.91	—	1.66	4.44	0.94	—	2.71	5.25	1.91	—
	2.74	6.19			1.94	4.92			3.17	6.45		
Cultivation in pots in 1995	1.79	4.82			1.21	3.36			1.78	3.81		
	2.38	5.61	2.43	—	1.63	4.32	0.83	—	2.38	4.82	2.01	—
	2.64	6.14			2.09	4.94			2.92	6.12		
Cultivation on the ground in 1996	1.82	4.48			1.41	3.50			3.01	4.39		
	2.40	5.67	2.34	6.08	2.06	5.01	1.61	5.05	3.30	5.20	3.65	7.58
	2.77	6.43			2.48	5.80			3.67	5.89		

Multiple values show the minimum, the mean, and the maximum values respectively. Numerals of the weight and the yield show the mean value.

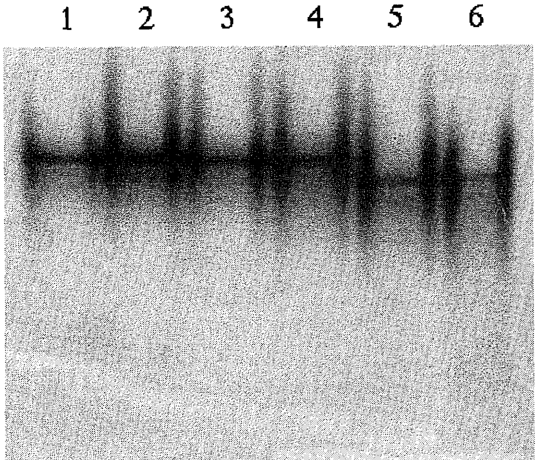


Fig. 8. Zymogram of SKD in *Senna* species.
1–2; *S. tora* type B. 3–4; *S. tora* type A. 5–6; *S. obtusifolia.*

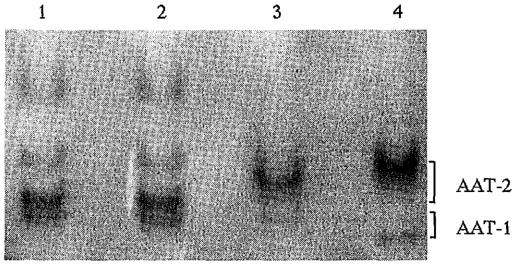


Fig. 9. Zymogram of AAT in *Senna* species.
1–2; *S. tora* type A. 3; *S. tora* type B. 4; *S. obtusifolia.*

S. obtusifolia type.

AAT (Fig. 9): The patterns of the bands were different between the three materials. The bands of AAT-1 showed the difference between *S. tora* (both types A and B) and *S. obtusifolia.* Those of AAT-2 showed the difference between the three materials.

Chromosome number

Chromosome numbers of the three materials (*S. tora* type A, type B, and *S. obtusifolia*) counted by T. Funamoto were all 2n = 26.

Discussion

We found two strains of *Senna tora* from Sri Lanka, which showed differences in morphology and allozyme electrophoretic pattern (both 2n = 26). *Senna tora* is widely distributed from tropical to subtropical regions throughout the Old World and Pacific regions. There must be many differing strains in the world as it is widely cultivated for its medicinal properties. It is necessary to do further research surveys on *S. tora* from many other places, and to clarify their botanical characteristics, so that those with best quality for medicine will be identified for circulation in the traditional medicine market. It is, however, also necessary to evaluate its pharmaceutical activities in detail.

The number and position of glands in the leaves is a key factor to distinguish *S. obtusifolia* from *S. tora*, but it showed different patterns in the early leaves even in the same plant.

The result of allozyme analysis showed, so far as the material examined, that *S. tora* type A, *S. tora* type B, and *S. obtusifolia* could be distinguished by the difference in the allele of AAT-2, and also that *S. tora* (both types A and B) and *S. obtusifolia* could be distinguished by the difference in the alleles of SKD and AAT-1. Further study of allozyme or other molecular data will help to clarify the relationship.

We are grateful to Dr. Tsuneo Funamoto for the identification of the chromosome number of the materials, and to Dr. Tatsuyoshi Morita for his kind advice on the allozyme analysis. We are also grateful to Dr. Michio Takido for many suggestions on *Senna tora*.

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1993年と1994年にスリランカで大きさの異なる2種類の *Senna tora* (L.) Roxb. の種子を入手して栽培したところ，形態的な違いを示した．そこで，これらの形態的特徴を詳細に検討し，遺伝的関係を明らかにする目的で比較栽培を行った．その結果，スリランカには，形態的および遺伝的に異なる2系統の *S. tora*（便宜上，type A と type B と称する）が存在することが明らかとなった．またアロザイム分析の結果，*S. tora* type A と type B は，AAT-2 の対立遺伝子の違いにより区別でき，両者（*S. tora* type A と type B）は，SKD および AAT-1 の対立遺伝子の違いにより *S. obtusifolia* と区別できた．

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